RESEARCH ARTICLE

Macromolecular Bioscience
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# Synthesis and Characterization of Cationic Hydrogels from Thiolated Copolymers for Independent Manipulation of Mechanical and Chemical Properties of Cell Substrates

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**Cells sense both mechanical and chemical properties in their environment and respond to these inputs with altered phenotypes. Precise and selective experimental manipulations of these environmental cues require biocompatible synthetic materials, for which multiple properties can be fine-tuned independently from each other. For example, cells typically show critical thresholds for cell adhesion as a function of substrate parameters such as stiffness and the degree of functionalization. However, the choice of tailor-made, defined materials to produce such cell adhesion substrates is still very limited. Here, a platform of synthetic hydrogels based on well-defined thiolated copolymers is presented. Therefore, four disulfide crosslinked hydrogels of different composition by free radical polymerization are prepared. After cleavage with dithiothreitol, four soluble copolymers P1–P4 with 0–96% cationic monomer content are obtained. P1 and P4 are then combined with $PEGDA_{3500}$ as a crosslinker, to fabricate 12 hydrogels with variable elasticity, ranging from 8.1 to 26.3 kPa and cationic group concentrations of up to 350 µmol $cm^{-3}$. Systematic analysis using COS7 cells shows that all of these hydrogels are nontoxic. However, successful cell adhesion requires both a minimal elasticity and a minimal cationic group concentration.**

## 1. Introduction

Tailor-made hydrogels are widely used in many biomedical applications, including drug delivery,[1,2] wound dressings,[3] tissue engineering,[4] and as substrates for in vitro cell cultivation.[5,6] The latter application has recently attracted much attention in stem cell research, as well-defined materials are needed to generate and maintain large numbers of cells in a nondifferentiated state or to provide materials that allow reproducible differentiation into defined lineages.[7–9] Tailor-made hydrogel substrates have important advantages over natural sources, as they can be synthesized rather easily with well-defined composition, stiffness, and functionalities[10,11] and enable cultivation of cells on top of a hydrogel film.[6] Furthermore, although natural-polymer-based hydrogels such as alginates, fibrin, and collagens are widely used due to their excellent biocompatibility and commercial availability,[5] potential drawbacks include low stiffness, limited long-term stability, limited options to modify material properties, batch-to-batch variability, and pathogen transmission.[6,12] Synthetic polymers on the other hand can be easily prepared on a larger scale in a reproducible manner with customized functionalities. The most widely used approach is based on soluble precursor copolymers with complementary reactive functional groups.[13–15] However, the usage of toxic metal catalysts or the complex, multistep synthesis of functional monomers limits the applicability of such bio-orthogonal coupling reactions in a biological application.[16–20] Consequently, only polyacrylamide and polyethylene glycol (PEG)-based hydrogels are commercially available as synthetic polymers, which explains their widespread application.[6] Polyacrylamide-based hydrogels can be easily fabricated from acrylamide and *N,N'*-methylene bis(acrylamide) with tunable mechanical properties and can be modified with peptides or proteins.[10,21] However, due to the in situ fabrication of the polymer network, characterization of the chemical composition is challenging. PEG enables more flexibility for fine-tuning and can be employed as a substrate for 2D and 3D cell culture.[22,23] Moreover, various kits are available that offer distinct crosslinking chemistry (Michael addition or photochemistry) and various types of functionalization in the gels (e.g., modified with the fibronectin-derived RGD-peptide or a degradable crosslinker). Crosslinking and thus hydrogel formation can be achieved under very mild conditions, however,

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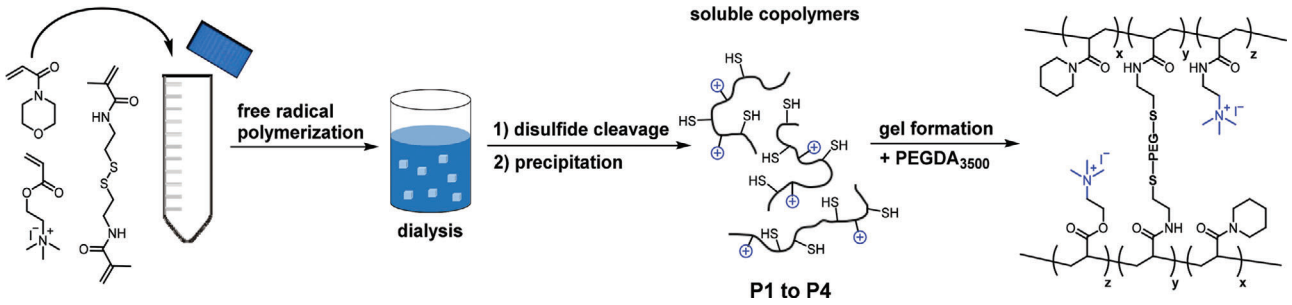
**DOI: 10.1002/mabi.202100453**




**Scheme 1.** Schematic representation of the newly developed hydrogel platform based on functionalized thiol-containing prepolymers crosslinked with **PEGDA$_{3500}$** via a Michael-addition reaction.

introduction of functional groups is limited since PEG carries the functional groups at the $\alpha,\omega$-end of the PEG chains.[24] In addition, gaseous ethylene oxide polymerization requires special equipment to minimize the risk of poisoning, even for laboratory-scale polymerizations, making it difficult to develop novel PEG-based polymers beyond those that are commercially available.[25]

In the last decade, thiolated polymers have attracted considerable interest due to their potential applications in controlled drug delivery as well as the formation of hydrogels for regenerative medical applications.[26–28] Recently, much progress was achieved in various thiol-related chemistries, in particular radical thiol-ene[29] and thiol-yne[30] chemistries as well as thiol-Michael addition to activated double bonds.[31] However, the efficient introduction of thiol functionalities into macromolecular systems still remains a major challenge. Therefore, thiol groups are typically incorporated into polymers in a protected form.[32,33] These protected thiols include thioesters,[34] thioethers,[35] and photolabile protecting groups, which are typically used in peptide synthesis.[36] Thiolactons, on the other hand, which allow by-product-free thiol formation in the presence of primary amines[37] have found broader application in materials science.[38] However, the most important approach of introducing thiols into polymers is still based on disulfide groups as protecting groups and has been used for monomers and initiators likewise. The release of the thiol groups can occur by using reducing agents such as dithiothreitol (DTT), or by thiol exchange reactions with other thiols.[39] One of the main applications of the disulfide group is the formation of disulfide crosslinked nanoparticles,[40,41] microcapsules,[42] and microspheres[43] for drug delivery in a reducing microenvironment. However, few reports have investigated the preparation of hydrogels as substrates for cell culture using thiolated copolymer precursors.[28]

Here, we have developed a versatile approach for the application of thiolated copolymers in hydrogel formation as cell substrates, which is scalable and compatible with various functional groups. First, we prepared hydrogels by free radical polymerization using *N,N'*-bis(methacryloyl)cystamine (BMAC) as a crosslinker, *N*-acryloyl morpholine (AMor) as hydrophilic monomer, and 2-acryloyl trimethylammonium ethyl iodide (TMAEA) as a cationic comonomer to facilitate positive charge-driven cell adhesion. These hydrogels were then carefully purified by dialysis to remove residual monomers before disulfide cleavage was carried out with DTT. The soluble, thiolated copolymers were then characterized by $^1$H NMR spectroscopy, SEC, and Ellman's assay to quantify their exact composition, molar mass, and thiol content. Next, hydrogels of different stiffness and concentration of the cationic moiety were prepared from these thiolated copolymers by crosslinking with **PEGDA$_{3500}$** via the Michael addition reaction (**Scheme 1**). Using COS7 cells, the biocompatibility and critical stiffness and cationic group concentration essential for cell adhesion were determined.

## 2. Experimental Section

### 2.1. Materials

All chemicals were obtained from local distributors and used without further processing unless otherwise stated. The reactions were carried out at room temperature unless otherwise stated. AMor and *N,N*-dimethylacrylamide were purified by distillation in vacuo prior to use. $^1$H NMR spectra were recorded with the FT-NMR spectrometer type AVANCE-III HD from Bruker Bio Spin at 400 MHz. The molar mass and dispersity of the polymers were analyzed by a custom-made SEC-system containing the following components: L-5000 LC Controller, 655A-11 Liquid Chromatograph (both Merck Hitachi), Smartline 2300 RI detector (Knauer), column oven (set to 60 °C, Knauer) PSS GRAM column set (1x precolumn, 1 × 1000 Å, 1 × 30 Å). Degassed *N,N*-dimethylformamide (DMF, HPLC grade) containing 5 g L$^{-1}$ LiBr was used as eluent and polymethylmethacrylate (PMMA) standards (PSS) were used for calibration. A UV-6300PC spectrometer (VWR) was used for UV/Vis measurements. Rheological measurements were performed with a Gemini advanced rheometer (Bohlin Instruments).

### 2.2. Characterization of the Polymers

#### *2.2.1. Nuclear Magnetic Resonance Spectroscopy (NMR)*

The compositions of the water-soluble copolymers were analyzed by $^1$H NMR spectroscopy by calculating the ratio between the integrals normalized to a proton of the methyl group of BMAC at 0.70–0.89 ppm, the ring protons of Amor at 3.10–3.90 ppm, and the protons of the methyl groups next to the quaternary amino group of TMAEA at 3.20–3.35 ppm, respectively.

#### *2.2.2. Ellman's Assay*

The thiol content was determined by Ellman's assay as previously reported.[39,44] For this purpose, 125 µL polymer stock solutions

(1 mg $mL^{-1}$ in 0.1 M phosphate buffer, pH 8) were mixed with 25 µL Ellman's reagent solution (4 mg $mL^{-1}$ in 0.1 M phosphate buffer, pH 8) and 1.25 mL 0.1 M phosphate buffer in triplicates. The resulting solutions were incubated at room temperature for 20 min. Afterward the absorbance of the solutions was measured at 412 nm. The thiol content was calculated using the Lambert–Beer law with an absorbance coefficient of 14 150 L $mol^{-1}$ $cm^{-1}$.

### 2.3. Characterization of the Hydrogels

#### *2.3.1. Swelling Ratio*

After complete gelation, gels were swollen in 0.01 M phosphate-buffered saline (PBS)-buffer (pH 7.4) for 24 h and surface water residues were removed before weighing. The swelling ratio ($Q_m$) was determined by comparing the weight of the fully swollen gel with the dry weight using the following equation[45]

$$Q_m = \frac{m(\text{swollen}) - m(\text{dry})}{m(\text{dry})} \times 100\% \quad (1)$$

#### *2.3.2. Rheology*

To determine the storage modulus ($G'$), samples were prepared in 15 × 2 mm cylindrical molds using the method described below for gel synthesis. The modulus was determined at 37 °C by time sweep experiments (5 min) using a 20 mm plate–plate setup. The measurement parameters used were 1% elongation, a constant frequency of 1 Hz, and a normal stress of 70 g.[46] For better comparison, the storage modulus $G'$ was converted into the elastic modulus $E$ using the following equation:[47] $E = 2(1 + \nu) \cdot G'$ with $E$ = elastic modulus and $\nu$ = Poisson's ratio. The Poisson's ratio is assumed to be 0.5 for hydrogels,[48] which allows to simplify the equation further and gives: $E = 3 \cdot G'$.

### 2.4. Synthesis of Thiol-Containing Prepolymers by Free Radical Polymerization

Briefly, for **P1** (96/4), 5.42 g 4-acryloylmorpholine (38.40 mmol, 24 eq.) and 461 mg BMAC (1.60 mmol, 1 eq.) were dissolved in tetrahydrofuran ([M] = 2 M) in a 50 mL centrifuge tube and sealed with a septum. The solution was degassed with argon for 20 min. After degassing, 123 mg 2′-azobis(4-methoxy-2,4-dimethylvaleronitrile) (V-70) (0.40 mmol, 0.25 eq) was added and the solution was polymerized over night at 42 °C. Following polymerization, the stiff gel was cut into pieces and swollen in water for 2 days, ensuring that the water was changed three times per day.

### 2.5. Reductive Cleavage of the In Situ Formed Hydrogels and Purification

The swollen hydrogel pieces were carefully filtered and placed in a round bottom flask. To cleave the disulfide bonds, DTT (5 equivalents with respect to the amount of BMAC used) was dissolved in 2 mL of water and added to the hydrogel. After adjusting the pH to 8 with NaOH (2 M), the solution was stirred under argon until a clear solution was obtained. After complete dissolution, the pH was adjusted to 4 with HCl (5 M). Insoluble components were removed by filtration (glass fiber filter). For purification, the polymer was precipitated in a large excess (20-fold) of degassed and acidified (pH 3) isopropanol, dissolved in dioxane, and precipitated in diethyl ether again. After precipitation, the resulting polymer was dried in vacuo and analyzed by $^1$H NMR, SEC, and Ellman's assay.

### 2.6. Gel Preparation

For the preparation of the gels, stock solutions of the thiol-containing polymers and the crosslinker polyethylene glycol diacrylate (PEGDA) were prepared. The polymers were dissolved in 0.1 M PBS buffer solution and adjusted to a pH of 7.4. Considering the thiol loading, as well as the desired concentration of the hydrogels, aliquots of the stock solutions were mixed with 0.1 M PBS buffer solution and transferred to cylindrical molds. To ensure a complete reaction procedure, the gels were incubated for 12 h at room temperature. All gels were prepared with a ratio of 1 (prepolymer thiol groups) to 0.7 (PEGDA end groups).

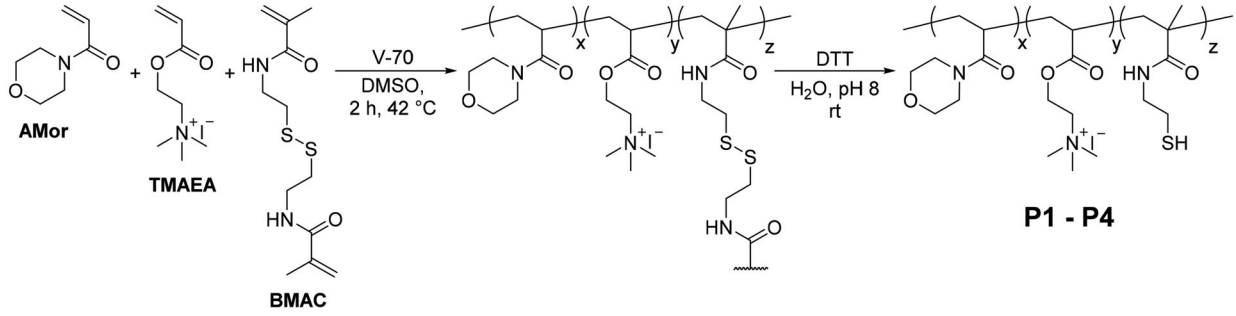
### 2.7. Cell Adhesion Assay

For all experiments with cells, gels with a volume of 200 µL were generated in the wells of µ-dishes (35 mm, high) from Ibidi. Gels were allowed to form for at least 2 h before cells were added. Subsequently, 100k cells in 2 mL growth medium were applied to each gel and incubated for 24 h at 37 °C and 5% $CO_2$. Cells were stained using the Live/Dead Cell Staining Kit II from PromCell which is based on the calcein acetoxymethylester (Calcein-AM) and Ethidium Homodimer III (EthD-III) dyes. Cell staining was performed according to the manufacturer's instructions. Briefly, cells were washed twice with serum-free PBS buffer. Next, 1.5 mL of a 10 × $10^{-6}$ M staining solution containing both Calcein-AM and EthD-III was applied to the samples and incubated for 45 min at room temperature. After this incubation, the staining solution was removed and cells were washed again with PBS buffer. The stained cells were observed using an EVOS FL microscope with 20x objectives and standard fluorescence filter sets.

## 3. Results and Discussion

### 3.1. Synthesis of the Thiol-Modified Copolymers

All polymer precursors were prepared by free radical copolymerization and in situ crosslinking based on a disulfide-containing crosslinker and AMor as hydrophilic comonomer. We preferred AMor over *N,N*′-dimethyl acrylamide due to the high solubility of the resulting copolymer in water (>50% w/v) and in a broad range of organic solvents.[29] As crosslinker, we selected BMAC which was synthesized via Schotten–Baumann reaction. To facilitate cell adhesion, the introduction of cationic functional groups into polymers was described previously.[49] Cell adhesion on typical, naturally occurring substrates is mediated by a specific biochemical interaction between the peptide motif RGD




**Scheme 2.** Synthesis of soluble prepolymers **P1**–**P4** by free radical polymerization.

**Table 1.** Analytical data of the copolymers **P1** to **P4**.

| Polymer | AMor/TMAEA/BMAC [mol%] | | $M_n$ [Da][b] | $Đ$[b] | Thiol content [mmol $g^{-1}$] | |
|---|---|---|---|---|---|---|
| | Theoretical | $^1$H NMR[a] | | | Calculated | Measured[c] |
| P1 | 96/0/4 | 95.9/0/4.1 | 21 200 | 2.28 | 0.546 | 0.574 |
| P2 | 64/32/4 | 64.6/31.6/3.8 | 17 900 | 2.52 | 0.424 | 0.398 |
| P3 | 32/64/4 | 41.1/55.2/3.7 | 14 300 | 2.18 | 0.317 | 0.287 |
| P4 | 0/96/4 | 0/95.8/4.2 | -[d] | -[d] | 0.292 | 0.297 |

[a] Determined via $^1$H NMR using the ratio of morpholine ring protons of AMor (3.31–3.90 ppm), the methyl protons next to the quaternary ammonium of the TMAEA (3.20–3.31 ppm) and the backbone methyl group protons of BMAC (0.70–0.89 ppm); [b] Determined via SEC using DMF + 5 g $L^{-1}$ LiBr as eluent and a PMMA-calibration standard; [c] Determined via Ellman's assay; [d] Polymers were not soluble in the SEC-solvent.

in extracellular matrix molecules and integrin receptors at the plasma membrane of cells. In contrast, cationic surfaces based on poly-L-lysine and poly(ethylene imine) coatings mediate cell adhesion primarily via the associated charge and are well-known substrates for cell cultivation.[50] To implement the properties of typical cationic surface coating, the cationic monomer 2-acryloyl trimethyl ammonium ethyl iodide (TMAEA) was copolymerized with AMor and BMAC according to the scheme described below (**Scheme 2**). The hydrogels were then cleaved by the addition of DTT and the soluble copolymers were isolated after precipitation in isopropanol with yields ranging from 72% to 93%.

We prepared three copolymers **P2** to **P4** with a content of 31.6 to 95.8 mol% of the cationic monomer TMAEA whereas **P1** served as a neutral reference material without any TMAEA. The copolymers were analyzed by $^1$H NMR spectroscopy, Ellman's assay, and SEC. **Table 1** summarizes the analytical results indicating excellent agreement of copolymer composition based on $^1$H NMR spectroscopy and Ellman's assay.

### 3.2. Gel Formation with PEGDA and the Michael–Thiol Reaction

#### *3.2.1. Hydrogel Properties—Stiffness and Swelling Behavior*

In addition to the chemical composition of the extracellular matrix, its mechanical properties also affect intracellular signals that influence cell behavior. To dissect these distinct, important inputs for studies of cell functions, it is crucial to have independent control over the chemical and mechanical biomaterial properties. The stiffness of the cell substrate is a particularly important mechanical input, which is transduced by intracellular signaling systems into changes of biochemical activity. This process, which is generally referred to as mechanotransduction, plays important roles in development and in cancer progression.[51,52] For meaningful studies of mechanotransduction, the stiffness of the cell substrate must be in the range, which the cells of interest typically encounter. The Young's moduli of most mammalian tissues range from 0.1 (brain) to 100 kPa (collagenous bone).[53] The stiffness of hydrogels produced by crosslinking thiol-modified prepolymers can be adjusted by varying the concentration of the polymers.[4,54] To prevent possible influences of the cationic component on the gel properties, the neutral polymer **P1** was adjusted accordingly. By crosslinking **P1** with **PEGDA$_{3500}$** in a concentration range from 20 to 300 mg $mL^{-1}$, we were able to obtain hydrogels with Young's moduli between 0.1 and 50 kPa (**Table 2**). All

**Table 2.** Data of the hydrogels prepared from **P1** and **PEGDA$_{3500}$** concerning their mechanical properties and their gelation time.

| $\beta$[a] [mg $mL^{-1}$] | $E$[b] [kPa] | $Q$[c] | $t_{gel}$[d] [min] |
|---|---|---|---|
| 20 | 0.1 ± 0.05 | - | 20 |
| 40 | 1.4 ± 0.15 | 20.1 ± 0.5 | 12 |
| 60 | 4.9 ± 0.02 | 13.4 ± 0.1 | 8 |
| 80 | 10.2 ± 0.24 | 10.8 ± 0.1 | 5 |
| 100 | 12.5 ± 0.32 | 9.7 ± 0.2 | 3 |
| 150 | 19.8 ± 3 | 8.5 ± 0.2 | 1.5 |
| 200 | 34.2 ± 1.2 | 7.2 ± 0.05 | 1 |
| 250 | 45 ± 3.2 | 6.5 ± 0.05 | 0.5 |
| 300 | 51 ± 6 | 6.1 ± 0.05 | 0.5 |

[a] Results from the combined mass of the thiol-containing prepolymers and the crosslinkers; [b] Determined in rheological oscillation experiments at 37 °C with a frequency of 1 Hz at 1% elongation with $E = 3G'$[48]; [c] Determined after 24 h of swelling at 37 °C in a 0.1 M PBS buffer; [d] Time of gelation determined with a tube inversion test.

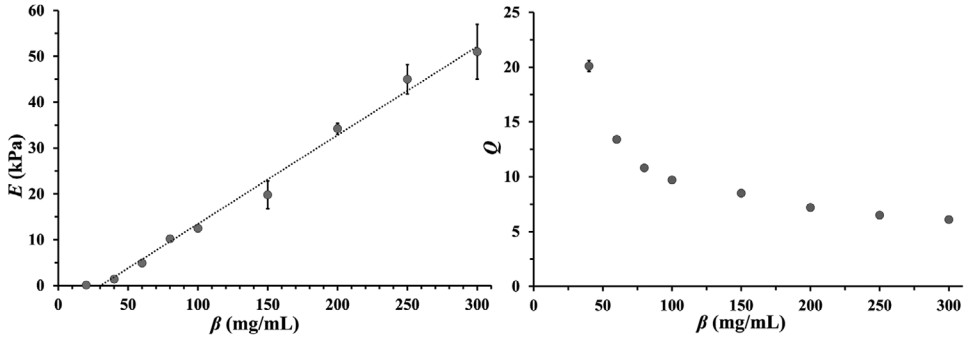



**Figure 1.** Left: Young's moduli of the **P1**/**PEGDA**$_{3500}$-hydrogels as a function of mass concentration; right: degree of swelling of the **P1**/**PEGDA**$_{3500}$ hydrogels as a function of mass concentration.

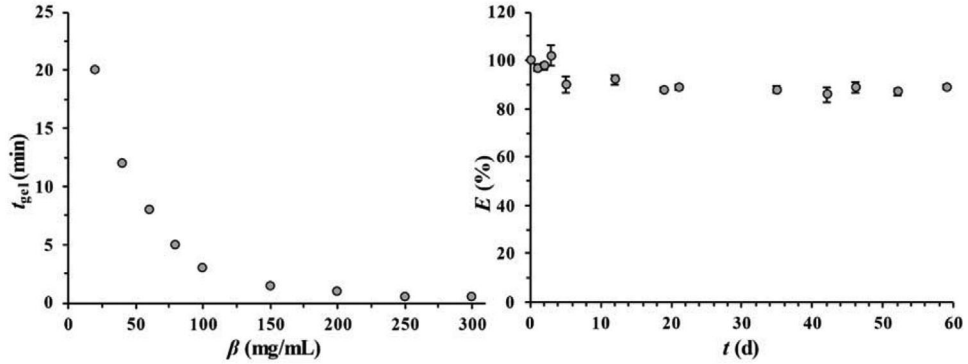



**Figure 2.** Left: Time of gelation of **P1**/**PEGDA**$_{3500}$ gels as a function of the gel concentration; right: Young's moduli of the **P1**/**PEGDA**$_{3500}$ gels ($\beta$ = 100 mg mL$^{-1}$) as a function of time (Table S1, Supporting Information).

gels prepared herein were transparent gels in the swollen state. Pictures of swollen, air-dried, and freeze-dried gel samples can be found in Figure S1 in the Supporting Information.

Moreover, the results show that with higher gel concentrations, a lower degree of gel swelling was observed, which presumably was due to the higher crosslinking density (**Figure 1**).

### 3.3. Hydrogel Properties—Gelation Time and Long-Term Stability

For the application of these hydrogels in 3D cell encapsulation experiments, it is very important that the crosslinking reaction occurs very rapidly. In particular, to prevent sinking of the cells to the bottom of the dish during the crosslinking reaction, the gelation time ($t_{gel}$) should ideally be in the range of a few minutes. The gelation time of the **P1**/**PEGDA**$_{3500}$ gels was determined via tube inversion tests (**Figure 2**).[55] The gels with the higher concentrations (80–300 mg mL$^{-1}$) showed fast gelation within a few minutes ($t_{gel}$ < 5 min), which is adequate to ensure an even distribution of cells in the gel. At lower polymer concentrations, gelation proceeded more slowly in the range of 8–20 min. To ensure consistent conditions for long-term cultivation of cells, the hydrogels should also remain stable under common culture conditions. To verify this for our system, rheological

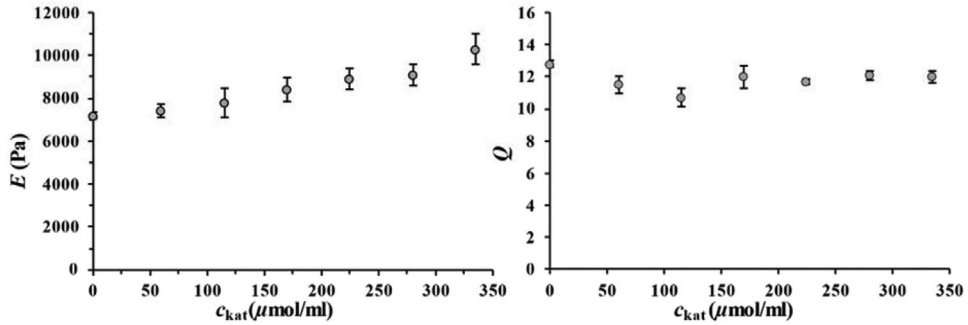



**Figure 3.** Left: Young's moduli of the hydrogels as a function of concentration of cationic units; right: degree of swelling of the hydrogels as a function of the concentration of cationic units; gels were prepared by crosslinking different mixtures of the polymers **P1** and **P4** with **PEGDA**$_{3500}$; hydrogel composition and values summed up in Table S2 in the Supporting Information.

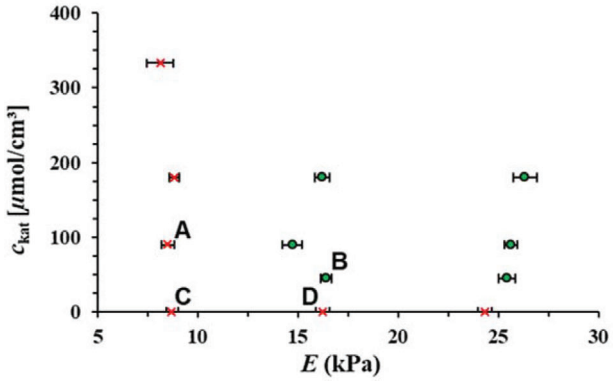



**Figure 4.** Investigation of cell adhesion as a function of the Young's modulus and the concentration of cationic groups in hydrogels; o = adhesion (green), x = no adhesion (red); gels were prepared by crosslinking different combinations of the polymers **P1** and **P4** with **PEGDA$_{3500}$** (Table S3, Supporting Information).

measurements of **P1/PEGDA$_{3500}$** gels (100 mg mL$^{-1}$, Table 2, entry 5) stored at 37 °C in 0.1 M PBS buffer were performed over a period of 2 months (Figure 2, right). The measurements showed no significant decrease in Young's modulus over the entire measurement period and the gels still showed almost 90% of the original stiffness at the end of the experiment.

### 3.4. Influence of the Cationic Functionalities

Next, we tested the effect of cationic groups on the mechanical properties of the gel. In general, it would be advantageous if the number of cationic units in the gels did not have a significant influence on their mechanical properties. To verify this, seven gels (75 mg mL$^{-1}$) prepared from **P1** and **P4** with a concentration of cationic functionalities ranging from 0 to 350 µmol mL$^{-1}$ were prepared and investigated with respect to their Young's modulus and swelling behavior (Table S2, Supporting Information and **Figure 3**). Especially in the concentration range from 0 to 175 µmol mL$^{-1}$, the gels showed only minor differences with respect to their Young's modulus as well as their swelling value. At very high concentrations of cationic functionalities ($c_{kat}$ = 335 µmol mL$^{-1}$), the stiffness of the gel increased by 43% compared to the neutral gels derived from **P1**. The swelling values of the tested gels showed only minor differences with values between 10.7 and 12.8.

### 3.5. Cell Adhesion Measurements

It has been shown for many different cell types that cell adhesion requires a minimal surface density of a functional group such as the RGD peptide or cations to enable cell adhesion.[56] Moreover, it is well known that many cell types sense substrate stiffness to achieve optimal cell adhesion to allow subsequent cellular processes such as cell migration, proliferation, or differentiation.[21] To determine the critical threshold for cell adhesion as a function of cationic group concentration and substrate stiffness, COS7 cells were used. Twelve gels were prepared based on mixtures of polymers **P1** and **P4** and crosslinked with **PEGDA$_{3500}$**. The resulting gels had a stiffness ranging from 8.1 to 26.3 kPa and cationic group concentration up to 350 µmol cm$^{-3}$ (see Table S3, Supporting Information). After a 24 h incubation period, cell morphology and cell adhesion to the gel surface were measured. As

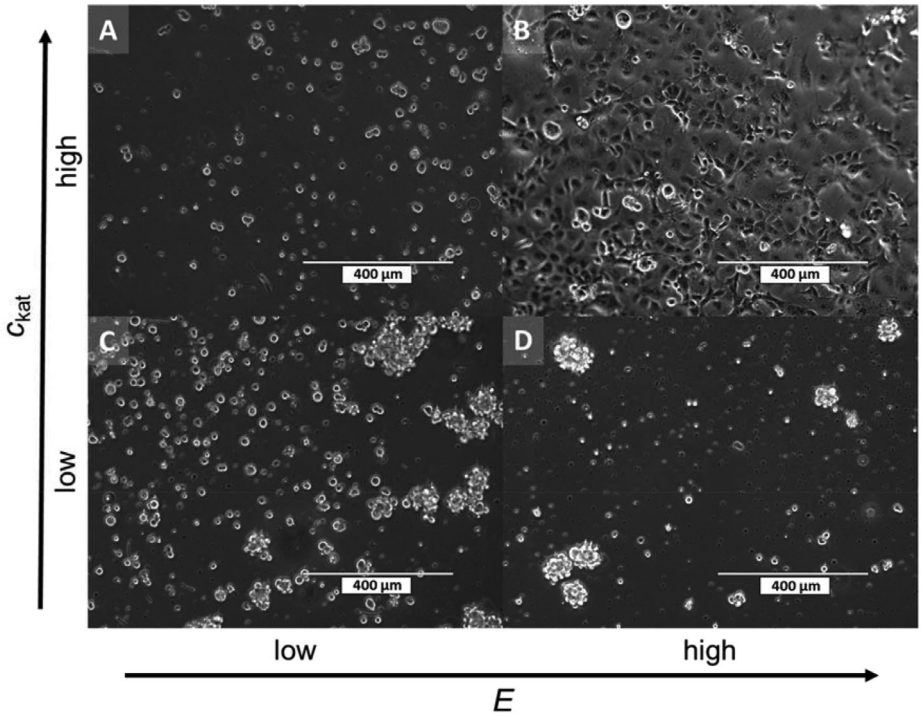



**Figure 5.** Representative images of COS7 cells on different hydrogels 24 h after cell seeding; properties of the hydrogels: A) $E$ = 8.5 kPa, $c_{kat}$ = 90 µmol, B) $E$ = 16.4 kPa, $c_{kat}$ = 45 µmol, C) $E$ = 8.7 kPa, $c_{kat}$ = 0 µmol, D) $E$ = 16.2 kPa, $c_{kat}$ = 0 µmol.

shown in **Figures 4** and **5**, COS7 cells did not adhere to the gel surface below a critical gel stiffness of $E \sim 14.7$ kPa, regardless of the concentration of cationic groups. Regarding the concentration of cationic functionalities, even a comparatively low concentration of 45 μmol $cm^{-3}$ enabled COS7 cell adhesion to the gel surface (Figure 5D). Representative images of the COS7 cells for the different adherent and nonadherent gel surfaces can be seen in Figure 5. No significant improvements in adhesion were observed when higher cationic concentrations were used, up to 335 μmol $mL^{-1}$.

## 4. Conclusion

We have developed a well-defined synthetic hydrogel platform with adjustable mechanical and chemical properties. Four copolymers **P1**–**P4** of different compositions were prepared and characterized by $^1$H NMR spectroscopy, SEC, and UV-Vis. Hydrogels were obtained by crosslinking these prepolymers with $\mathbf{PEGDA_{3500}}$. The resulting hydrogels were biocompatible and we demonstrated that a minimum amount of cationic moieties and a certain gel stiffness were required for COS7 cell adhesion. Moreover, these gels allowed to analyze the impact of cationic group concentration independent from gel stiffness especially in the concentration range from 0 to 175 μmol $mL^{-1}$, where only minor differences with respect to their Young's modulus as well as their swelling value were detected. We believe that this hydrogel system is particularly useful for experiments in which the cellular phenotype is studied as a function of stiffness and concentration of cationic charges and therefore represents a versatile alternative to poly-L-lysine or poly-L-ornithine coatings.

## Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

## Acknowledgements

N.S. is grateful for financial support by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation, grant number: 397037958).
Open Access funding enabled and organized by Projekt DEAL.

## Conflict of Interest

The authors declare no conflict of interest.

## Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Keywords

cell adhesion, COS7 cell, hydrogel, Michael reaction, precursor polymer

Received: November 10, 2021
Revised: January 17, 2022
Published online: February 20, 2022

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